

THE EFFECT OF A LIVE CULTURE OF *B. PRODIGIOSUS* ON TRANSPLANTED TUMORS IN EXPERIMENTAL ANIMALS

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As early as the last century, attempts were made to treat malignant new growths by the utilization of microorganisms and their products. N. F. Gamaleya [1,3] was the first to propose this toxitherapy of cancer in the environment of the oncological clinic, utilizing for his goal the toxins of a killed culture of *B. prodigiosus*.

Later many investigators [2,4,6,7,8 and others] studied the influence the toxins of this microbe had upon various cancers, both in the clinic and experimentally. Many of them [4,5,6] succeeded in suppressing the growth of experimental tumors in animals by introducing into them the toxins from *B. prodigiosus* cultures; there being seen a picture of hemorrhages into the tissues of the growth itself.

The next stage in the study of this problem consisted in attempts to fractionate the cultures of this microbe in such a manner as to isolate that *B. prodigiosus* fraction which was active against the cancers [9,10]. Still, the whole question of the therapeutic effectiveness of *B. prodigiosus* cultures and its various fractions has remained open.

In developing experimental methods of complex bio-immunological therapy of malignant new growths, we set ourselves the task of investigating the influence exerted by a living culture of *B. prodigiosus* upon tumor transplants, inasmuch as we are aware of only a single study devoted to this question [4].

METHODS AND RESULTS

In these experiments we used white rats, males weighing 250-280 g and male chinchilla breed rabbits weighing 2.5-3 kg each.

M-1 sarcoma was injected subcutaneously into the rats in the form of a 10% suspension, the dose being 0.25 ml; 20% suspension of Brown-Pierce tumor was injected intratesticularly to the rabbits, the dose being 0.5 ml. Among the factors needing consideration as to the effect of living cultures of *B. prodigiosus* upon tumor growth, was the obtaining by us of three fresh strains (Numbers 1, 8 and 10) of living *B. prodigiosus* from the L. A. Tarasovich Central State Institute of Scientific Standards. We conducted preliminary titrations with these cultures as to their toxicity.

Experiments proved that a single intraperitoneal injection, into rats of 400 million microbes per 1000 g of body weight and, into rabbits, of 1.5 billion microbe bodies per 1,000 g of body weight, had but little effect upon the animals. If the injections were repeated at 3 day intervals, both the rats and the rabbits did lose a little weight but otherwise withstood the inoculations with *B. prodigiosus* quite well. The rats had less of a reaction than the rabbits which would develop a fever, lose their appetite and not return to a normal state for

2-3 days. The first experiments were performed on rats for a comparative study of the influence exerted by the three living strains of *B. prodigiosus* on sarcoma M-1. 50 rats, all of about the same age and weight, were used in the first experimental series. Commencing with the 9th day after the subcutaneous inoculation of tumor M-1, three animal groups (10 in each) received intraperitoneally the corresponding strain of *B. prodigiosus*.

As controls, we had an animal group which received intraperitoneally saline solution only. The size of the tumor was measured by using the formula for the area of an ellipse. After giving 5 injections at 3 day intervals, i.e., on the 15th day after beginning the injections, the rats were sacrificed and the tumors excised and weighed.

In further experiments we examined the influence exerted by a living *B. prodigiosus* culture, strain Number 10, upon the capacity of the Brown-Pierce tumor to metastasize. 11 rabbits were used, 6 being injected and 5 serving as controls. On the 9th day following the intratesticular introduction of the tumor suspension, the rabbits received intravenously 700 million microbe bodies of this culture per 1 kg body weight. After that, the injections were given intraperitoneally at 3 day intervals. A total of 7 injections was given.

The results of the experiments conducted on the rats in order to observe the influence of living *B. prodigiosus* cultures on sarcoma M-1 are shown in Table 1.

TABLE 1

Influence of Living Culture of *B. Prodigiosus* (by intraperitoneal injection)
Upon the Development of Sarcoma M-1

Strain	Number of animals		Average dimension of tumor (in cm ²)		Average weight of tumor after sacrificing the animals (in mg)
	used in exp.	perished during experiment	before injection	at end of exp.	
1	10	—	1.3	3.2	7.457
8	10	3	1.7	6.1	21.365
10	10	—	1.1	2.8	4.080
Control	20	1	1.0	11.5	28.619

From Table 1 it can be seen that the living culture of *B. prodigiosus* does have a somewhat inhibitory influence upon sarcoma M-1 growth, this being most marked when strain Number 10 was used. Analogous results were seen in the rats who received subcutaneous injections of strain Number 10 *B. prodigiosus* in the vicinity of the growing sarcoma M-1 (Table 2).

However, it should be stated that, in these experiments, we did not observe complete regression of the tumor as a result of the microbe having been introduced.

Microscopic studies of the tumors removed from the rats receiving *B. prodigiosus* injections, revealed large zones of tumor necrosis extending from the center to the periphery of the growth, there being seen, along with well growing areas of malignancy, masses of degenerating cancer cells having markedly abnormal nuclear structures. Quite often, even in the healthy cancer areas, there could be seen extensive focal hemorrhages on the edges of which there could be seen beginning necrosis being walled-off by a leucocytic barrier. In individual instances, the hemorrhages would involve the greater portion of the tumor capsule. Such microscopic observations could be made in the tumors of the control animals.

The influence exerted by live cultures of *B. prodigiosus* upon the malignant Brown-Pierce tumors is seen when Table 3 is examined.

Table 3 shows that, of the 6 experimental animals there was full tumor recession in 4 rabbits, the testicular growth dissolving completely. These rabbits stayed well for 8 months after being inoculated with the Brown-Pierce tumor. The remaining rabbits of this group perished at various times from the beginning of the experiment. Rabbit Number 2298 died on the 32nd day after being inoculated with the tumor as a result of

TABLE 2

Animal group	Animals		Average dimension of tumor (in cm ³)		Average weight of tumor after sacrificing the animals (in mg)
	used in exp.	dying during exp.	before injection	at end of experiment	
Experiment	6	—	1.5	3.8	9.042
Control	5	—	1.4	15	38.750

multiple metastasis. These were especially numerous in the epiploic lymphatics, in the loops of the large and small intestine and in the mesentery. Rabbit Number 1095 died 150 days after being inoculated with the Brown-Pierce tumor. At autopsy, there was found a 5x3 cm tumor in the side of the left seminal cord. This cancer metastasis was an enormous necrotic mass with massive liquefaction of the tumor tissue itself. It should be noted further that this rabbit had areas of focal inflammation in the central portions of both lungs there being pronounced carnification of pulmonary tissues. All the control animals died on the 30-32nd day after being injected with the Brown-Pierce tumor.

TABLE 3

Animal group of rabbits	Used in exp.	Tumor took	Dying during exp. period by 32d day	Tumor dissolved	Surviving to 8th month and longer
Experiment	6	6	1	4	4
Control	5	5	5	—	—

Summarizing, we can state that living cultures of *B. prodigiosus* exert a varying suppressing effect upon rat sarcoma M-1, the different strains of the microbe exhibiting variations in their effectiveness. The antitumor effect of the living microbe is seen very strikingly in the fact that the Brown-Pierce tumor was seen to fully regress and dissolve in the majority of animals.

SUMMARY

Intraperitoneal inoculation of three different strains of live cultures of *B. prodigiosus* led to a varying regression of rat sarcoma M-1 injected subcutaneously. In rabbits inoculated intratesticularly with a Brown-Pierce tumor, there was observed a complete destruction of the malignancy, the large majority of the animals surviving for 8 months and more.

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